CLINICAL STUDY

Transfer of meropenem in the ex vivo human placenta perfusion model

MICHAEL HNAT & ROGER E. BAWDON

Division of Maternal-Fetal Medicine, Department of Obstetrics and Gynecology, The University of Texas, Southwestern Medical Center at Dallas, USA

Abstract

Objectives. To determine maternal-fetal transplacental passage of meropenem in the ex vivo human perfusion model.

Study design. Term placentae ($n = 6$) were collected immediately after delivery. A single cotyledon was localized, perfused and stabilized with physiologic Eagles minimal essential medium containing 3% bovine albumin and heparin as described by Chaliel (Chaliel JC. Criteria for evaluating perfusion experiments and presentation results. *Contrib Gynecol Obstet* 1985; 13:32–39). Meropenem was added to the maternal medium in concentrations similar to maternal serum peak and trough levels, then perfused through the maternal circulation of the cotyledon. To assess transfer and accumulation, fluid aliquots from both the maternal and fetal compartments were collected over an hour at defined intervals in an open and closed system. Antipyrine ^{14}C was added to the medium in order to calculate the transport fraction and clearance indexes. Meropenem and antipyrine ^{14}C concentrations were determined by High-pressure Liquid Chromatography and liquid scintillation, respectively.

Results. Mean antipyrine transport fraction was 2.33 ± 0.25 . Maternal and fetal mean meropenem peak concentrations were $54.3 \pm 3.3 \mu\text{g/ml}$ and $2.2 \pm 0.18 \mu\text{g/ml}$, respectively. Whereas, maternal and fetal mean trough concentrations were $12.7 \pm 1.3 \mu\text{g/ml}$ and $0.41 \pm 0.10 \mu\text{g/ml}$, respectively. Mean peak clearance index was 0.077 ± 0.007 and the mean trough was 0.052 ± 0.015 . Mean accumulation for the peak and trough concentrations of meropenem were 0.9 and 2.95 $\mu\text{g/ml}$, respectively.

Conclusions. Transplacental passage of meropenem was incomplete in the ex vivo human placental perfusion model. Accumulation was also noted in the fetal compartment

Keywords: Meropenem, antibiotic, fetus, placental perfusion and pregnancy

Condensation

Incomplete transplacental passage and accumulation of meropenem were noted in the ex vivo human placental perfusion model.

Introduction

Meropenem is a synthetic carbapenem β -lactam antibiotic with a broad antimicrobial activity against gram-negative, gram-positive and anaerobic microorganisms. It is as effective as imipenem/cilastatin and is more effective than cephalosporin-based combination in the treatment of a range of serious infections. Resistance to meropenem is uncommon [1–3].

Use of this particular antibiotic is increasing secondary to microbial resistance to other antibiotics. In addition, meropenem has been administered to pregnant women. Like many other medications, information regarding pharmacokinetics is limited for meropenem in pregnancy [4,5]. Our study objective was to quantify the maternal to fetal transplacental passage of meropenem in the ex vivo human placental perfusion model.

Material and methods

Term placentae were collected immediately after delivery from either cesarean sections or vaginal deliveries in accordance with the University of Texas

Correspondence: Michael Hnat, D.O., UT Southwestern Medical Center, 5323 Harry Hines Blvd., Dallas, TX 75390-9032, USA. Tel: 214/648 4746.

Fax: 214/648 4763. E-mail: michael.hnat@utsouthwestern.edu

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Southwestern Medical Center Institutional Review Board for Human Studies' guidelines and were transported to the laboratory in normal saline immediately after delivery.

The single cotyledon placental perfusion system was used as described by Chaliier [6]. A fetal artery and vein on the chorionic plate was cannulated with 3.0F and 5.0F catheters, respectively (Figure 1). The fetal circulation of the selected cotyledon was gently perfused with Eagles minimal essential media plus 3% bovine albumin and 1.0 units/ml of heparin (Sigma Chemical Co., St. Louis, MO).

The localized cotyledon and surrounding placental tissue were transferred to a temperature-controlled chamber (37°C) where the fetal circulation was perfused in a closed-system at a rate between 4.5 and 5 mL/min for 20 minutes to remove any residual blood and to stabilize the perfusion pressure between 35 and 45 mmHg (Figure 2). Prior to perfusing, two 18-gauge needles were inserted into the intervillous space of the cotyledon to reestablish the maternal circulation (Figure 3). Maternal flow rate was 17 mL/min. Those placental cotyledons, which failed to reach a stable baseline pressure or found to have a vascular leak, were discarded.

The maternal and fetal compartments consisted of 150 mL of Eagle's minimal essential medium (pH 7.2 – 7.4), which were aerated with 95% oxygen and 5% carbon dioxide and were continually mixed by a magnetic stirring bar. Transfer studies were performed with meropenem (Zeneca Pharmaceuticals, Wilmington, DE). Meropenem was added to the maternal medium in concentrations similar to serum peak and trough levels. Experiments were first conducted in an open-open system to determine transport fraction, then in a closed-closed recirculation system to determine accumulation. The clearance index of meropenem was also calculated. Fluid aliquots from both the maternal and fetal compartments were collected for analysis.

A total of 6 placentae met criteria to be included in the study. A trough transfer study for meropenem

was performed in one placenta and a peak study in another. Both, peak and trough transfer studies were conducted in the remaining four. Studies were completed first in an open system for 1 hour and then in a closed system. For example, in the 4 placentae, trough studies were performed in an open system and then in a closed system, followed by peak studies in an open system and then in a closed one.

Lastly, transport fraction and clearance indexes were determined for each placenta by means of the ^{14}C antipyrine reference method of Chaliier [6]. Placentae were studied in a nonrecirculating (open) system for 60 minutes to determine transport fraction of ^{14}C antipyrine. Greater than 40% maternal-fetal transfer of antipyrine is an indication of a successful maternal-fetal match.

Meropenem and antipyrine ^{14}C concentrations were measured by High-pressure Liquid Chromatography (HPLC) and liquid scintillation, respectively. Reproducibility studies on the meropenem assay were performed.

The HPLC analysis of meropenem was carried out on all Waters Associate instruments (Medford, MA). The meropenem standards were prepared from laboratory assay powder provided by Zeneca Pharmaceuticals. The HPLC assay of meropenem consisted of preparation of a standard curve to breach the therapeutic range of the drug. All specimens and standards were extracted in aliquots of 0.5 mL. To each tube was added 2.5 mL of acetonitrile to precipitate blood, serum and other proteins. All samples were centrifuged at $800\times g$ for 10 minutes and the supernatant was decanted into a clean 10 mm x 100 mm screw-capped test tube. Five milliliters of methylene chloride was then added to each tube. The tubes were mixed on a vortex mixer and were again centrifuged at $800\times g$. The upper aqueous layer was removed and placed in HPLC micro tubes and placed in a Waters 717 + autosampler. An injection of 20 mL was made. The HPLC mobile phase consisted of 95% mmole ammonium acetate and 5% acetonitrile (pH 4.0).

Figure 1. Fetal circulation. (a) Cannulization of vein. (b) Establishing fetal circulation.

Figure 2. Placental perfusion model.

Figure 3. Maternal circulation. (a) Perfused cotyledon. (b) 18 gauge probes for maternal perfusate.

The HPLC column used was Waters μ -Bond a-Pak C_{18} with a flow rate of 1.5 mL/min. The HPLC pump was a Waters model 510. The Waters 481 detector settings were a wavelength of 298 nm with a sensitivity setting of 0.01–0.05 absorbance units. The retention time was about 7 minutes and the peak height was recorded on a Kipp and Zonan (Holland) 10 mV recorder. Using least squares regression analysis and the correlation coefficient of the standard curve all specimens were quantified and reported. Reproducibility studies were done on meropenem over a 3 day period to determine the within batch and between batch reproducibility.

Results

For the HPLC determination of meropenem concentrations, reproducibility studies provided values

with a minimum sensitivity of 0.2 μ g/mL; and, a within batch and between batch reproducibility of > 90%. Maternal and fetal Meropenem concentrations for the maternal and fetal perfusate are listed in Table I. For the maternal perfusate, the mean peak and trough levels and standard error were 54.3 μ g/mL $\pm$ 3.3 and 12.7 $\pm$ 1.3 μ g/mL, respectively and for the fetal, they were 2.2 $\pm$ 1.8 μ g/mL and 0.44 $\pm$ 0.10 μ g/mL. The clearance index and accumulation for both peak and trough levels are shown in Table II. The mean transport factor for antipyrine 14 C was 2.33 $\pm$ 0.25.

Discussion

The pharmacokinetics and pharmacodynamics of meropenem have been described in patients who are healthy or critically ill, who have renal or hepatic

Table I. Meropenem concentrations.

Placenta #	Peak Concentration ($\mu\text{g/mL}$)		Trough Concentration($\mu\text{g/mL}$)	
	Maternal	Fetal	Maternal	Fetal
1	63.7	1.97	17.1	0.68
2	53.3	2.80	12.1	0.57
3	—	—	13.0	0.5
4	59.8	1.80	12.3	0.36
5	45.8	2.45	9.1	0.10
6	49.0	2.17	—	—

Table II. Meropenem clearance index and accumulation.

Placenta #	Clearance Index		Accumulation ($\mu\text{g/mL}$)	
	Peak	Trough	Peak	Trough
1	0.062	0.078	3.3	1.0
2	0.062	0.067	3.5	1.4
3	—	0.011	—	1.5
4	0.085	0.082	5.9	0.7
5	0.096	0.020	2.8	0.7
6	0.082	—	5.4	—

impairment and who are elderly or young [2,7,8]. However, little is known regarding its pharmacokinetics and safety in the mother and the fetus during pregnancy [4,5]. Meropenem is listed as Category B in pregnancy [5]. In other words, no adequate or well-controlled studies have been carried out in pregnant women. No fetal harm was noted in rats and cynomolgus monkeys at doses of 1.8 and 3.7 times the human dose [5]. One abstract was available that described the efficacy and safety of meropenem during pregnancy ($n=13$) and during the puerperal period ($n=11$) [9]. Our study provides initial data regarding transplacental passage of meropenem in the ex vivo human model.

Meropenem is similar in structure to imipenem, another carbapenem beta-lactam antibiotic. Imipenem's pharmacokinetics is known to change during pregnancy [8]. When compared to healthy, non-pregnant women, Imipenem's volume of distribution was larger, clearance was accelerated and peak levels were significantly lower in those who were pregnant [8]. Meropenem mean values for peak plasma concentrations and volume of distribution after 1 g doses are known for a healthy individual and are 54.8 to 61.6 $\mu\text{g/mL}$ and 12.5 to 23 L, respectively [3]. These values during pregnancy are unknown.

Heikkilä and co-workers demonstrated transplacental passage of imipenem and noted therapeutic concentrations in the fetal compartments late in pregnancy [8]. In our study, transplacental passage of meropenem was noted to occur in the ex vivo model. However, concentrations of meropenem in

the fetal effluence were less than maternal levels and were below therapeutic levels. Therefore, placental transfer was incomplete. The low clearance index obtained in this study might be explained by the solubility and molecular weight of meropenem. Most drugs cross the placenta by simple diffusion. Rate of diffusion is dependent upon solubility, molecular weight and concentration of the free drug. Hydrophilic substances cross the placenta slower than lipophilic ones. Meropenem is hydrophilic with a molecular weight of 437.52 d [3]. Further research is needed in vivo to determine both fetal and maternal concentrations of meropenem.

Normal physiologic changes of pregnancy may alter the pharmacokinetics of meropenem not apparent in our ex vivo model. In pregnancy, blood volume and glomerular filtration rate increase 40–50%. Because meropenem and its inactive metabolite are excreted by the kidney through glomerular filtration and tubular secretion serum concentrations may be decreased in pregnancy [3,8].

Accumulation of meropenem in the fetal compartment was noted in the closed system. Minimal accumulation was noted at a maternal peak and trough concentrations. Fetal accumulation increased with maternal meropenem concentrations but remained below the therapeutic levels.

Our study suggests that meropenem crosses the placenta incompletely with lower fetal concentrations than maternal. In addition, the placental clearance and fetal accumulation of meropenem are low at both peak and trough levels. Further investigation of

meropenem in vivo is needed to determine both the maternal and fetal pharmacokinetics.

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